AGILENT TECHNOLOGIES, INC.
Legal Department, M/S DL429
Intellectual Property Administration
P. O. Box 7599
Loveland, CO 80537-0599

PATENT APPLICATION
ATTORNEY DOCKET NO. 10010372-1

OFFICIAL

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|---|---|---|
| In re Application of: **Nelson R. Holcomb et al.** | : | Date: December 20, 2002 |
| Serial No.: 09/900,084 Filed: July 5, 2001 | : | Group Art Unit: 1634 |
| For: A BUFFER COMPOSITION AND METHOD FOR HYBRIDIZATION OF MICROARRAYS ON ADSORBED POLYMER SILICEOUS SURFACES | : | Examiner: Arun K. Chakrabarti |

OFFICIAL AMENDMENT

Box Non Fee Amendment
Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

An Office Action mailed September 20, 2002 was received for the above-referenced patent application. Applicant respectfully requests consideration of the following amendment.

IN THE CLAIMS

Please replace Claims 1-57, as originally filed, with the following replacement Claims 20-35 and 50. Claims 32, 34, 35 and 50 have been amended, and non-elected Claims 1-19, 36-49 and 51-57 have been canceled, without prejudice. The amendments are reflected in the replacement claims herein below. Canceled Claims 1-19, 36-49 and 51-57 are not included in the replacement claims herein. The attached Appendix provides a marked-up version of the claims, as filed, and a request to amend Claims 32, 34, 35 and 50 and cancel Claims 1-19, 36-49 and 51-57. The status of the claims is shown in parenthesis at the beginning of each claim.

20. (NOT AMENDED) A method of hybridizing a microarray of oligonucleotides bound to an adsorbed polymer surface on a siliceous substrate with a nucleic acid material comprising the step of:

incubating the nucleic acid material with the microarray of oligonucleotides on the adsorbed polymer surface in a hybridization solution at a hybridization temperature ranging from about 55°C to about 70°C so as to hybridize the nucleic acid material,

wherein the hybridization solution comprises a buffer composition that comprises a pH within a range of pH 6.4 to 7.5, a non-chelating buffering agent that maintains the pH within the pH range, and a monovalent cation in a monovalent cation concentration ranging from about 0.01 M to about 2.0 M.

21. (NOT AMENDED) The method of Claim 20, wherein in the step of incubating, the non chelating buffering agent is selected from a group consisting of 2-[*N*-morpholino]ethanesulfonic acid (MES), 3-(*N*-Morpholine)propanesulfonic acid (MOPS), Piperazine-*N*,*N*'-bis(2-ethansulfonic acid (PIPES), Tris(hydroxymethyl)aminomethane hydrochloride (TRIS-HCl), Hydroxyethylpiperazine-*N*'-2-ethanesulfonic acid (HEPES), and *N*-Tris(hydroxymethyl)methylglycine (TRICINE).

22. (NOT AMENDED) The method of Claim 20, wherein in the step of incubating, the monovalent cation is selected from a salt consisting of one or more of LiCl, NaCl and KCl and the monovalent cation concentration ranges from about 0.1 M to about 2.0 M.

23. (NOT AMENDED) The method of Claim 20, wherein the adsorbed polymer surface comprises a polycationic polymer.

24. (NOT AMENDED) The method of Clam 23, wherein the polycationic polymer is selected from a group consisting of one or more of polyethylenediamine, poly-acrylamide, poly-L-arginine, poly-L-histidine, and poly-L-lysine.

25. (NOT AMENDED) The method of Claim 20, wherein in the step of incubating, the buffer composition further comprises a chelating agent selected from a group consisting of one or more of ethylenediaminetetraacetic acid (EDTA), trans-1, 2-diaminocyclohexanetetraacetic acid (CDTA) and diethylenetriaminopentaacetic acid (DTPA) that has a chelating agent concentration of less than about 100 μM.

26. (NOT AMENDED) The method of Claim 20, wherein in the step of incubating, the buffer composition further comprises an ionic surfactant selected from a group consisting of one or more of sodium dodecyl sulfate (SDS), lithium lauryl sulfate (LLS), N-lauryl sarcoside, acylated polypeptides, linear alkybenzene sulfonates, lignin sulfonates, paraffin sulfonates, sulfosuccinate esters, alkylnaphthalene sulfonates, isethionates, alkanolamine condensates, and N-alkylpyrrolidones, and wherein the step of incubating comprises using a hybridization chamber, and the ionic surfactant is provided in an amount sufficient to wet surfaces of the hybridization chamber and loosen bubbles impinged on the surfaces of the hybridization chamber.

27. (NOT AMENDED) The method of Claim 26, wherein the amount of ionic surfactant is a surfactant concentration ranging from about 0.01% to about 0.2% (w/v).

28. (NOT AMENDED) The method of Claim 20, wherein the buffer composition has a total cation concentration of about 0.02 M to about 2.0 M.

29. (NOT AMENDED) The method of Claim 20, wherein in the step of incubating, the non chelating buffering agent is 2-[*N*-morpholino]ethanesulfonic acid (MES), the monovalent cation is LiCl, the monovalent cation concentration is greater than or equal to 300 mM, the pH is within the range of pH 6.6 to 6.8.

30. (NOT AMENDED) The method of Claim 29, wherein in the step of incubating, the buffer composition further comprises one or both of a chelating agent ethylenediaminetetraacetic acid EDTA having a chelating agent concentration of about 50 μM, and an ionic surfactant selected from sodium dodecyl sulfate (SDS), lithium lauryl sulfate (LLS) having a surfactant concentration that ranges from about

0.02 % to about 0.1 % (w/v), and the buffer composition has a total cation concentration of about 750 mM.

31. (NOT AMENDED) The method of Claim 20, before the step of incubating, further comprising the step of combining the nucleic acid material with the buffer composition.

32. (AMENDED) The method of Claim 20, after the step of incubating, further comprising the step of interrogating the hybridized microarray at a first location, the first location being a physical location either where the incubation of the microarray is performed or another location separate from the microarray incubation location.

33. (NOT AMENDED) The method of Claim 32, further comprising the step of transmitting data representing a result of the interrogation.

34. (AMENDED) The method of Claim 33, further comprising the step of receiving the transmitted data at a second location, the second location being a physical location that is different from one or both of the first location where the microarray interrogation is performed and the microarray incubation location.

35. (AMENDED) The method of Claim 34, wherein the first location is remote from the second location, the remote first location being physically separated from the second location.

50. (AMENDED) A method of performing a high temperature hybridization assay comprising the step of:

incubating a nucleic acid material with a microarray of oligonucleotides in a hybridization solution at a hybridization temperature ranging from about 55°C to about 70°C so as to hybridize the nucleic acid material,

wherein the microarray comprises a siliceous substrate with an adsorbed polymer surface and oligonucleotides bound to the adsorbed polymer surface, and

wherein the hybridization solution comprises a pH within a range of pH 6.4 and 7.5 and a buffer composition, the buffer composition comprising a non-chelating buffering agent that maintains the pH within the range and a monovalent cation having a monovalent cation concentration ranging from 0.01 M and 2.0 M.

## REMARKS

The patent application was originally filed with Claims 1-57. Claims 1-57 were subject to a restriction requirement. An attorney of record, Gordon M. Stewart elected with traverse the invention of Group II, Claims 20-35 and 50 via a telephone interview with the Examiner on December 12, 2001. In the pending Office Action, the Examiner made the restriction final. Claims 20-35 and 50 were rejected in the pending Office Action. Applicant has amended Claims 32, 34, 35 and 50, and has canceled the non-elected Claims 1-19, 36-49 and 51-57, without prejudice herein. No new matter has been added. Reconsideration is respectfully requested.

The restriction requirement is traversed with respect to Claim 1 of Group I, as it relates to the elected invention in Group II, Claim 50. Claim 50 is directed to a method of performing a high temperature hybridization assay using the buffer composition of Claim 1, in Group I. However, Applicant has amended Claim 50 to incorporate the features of Claim 1, of Group I. Amended Claim 50 is now an independent claim. The amendment to Claim 50 does not narrow the scope any element recited in Claim 50 beyond that which was originally intended when Claim 50 was originally filed. As such, the election of Group II claims is affirmed if and when the Examiner enters the amendment to Claim 50.

Claims 1-19, 36-49 and 51-57 are canceled herein, without prejudice, for being drawn to a non-elected invention. Applicant reserves the right to file a continuation application with non-elected Claims 2-19, 36-49 and 51-57 during the pendency of the present application.

Claims 32-35 were rejected under 35 USC 112, second paragraph, as being indefinite. The Examiner contended that recitation of the word 'location' in the claims was unclear.

Applicant has amended Claims 32, 34 and 35 in accordance with Applicant's specification at page 26, lines 1-9, where it is described that a 'location' in this context is a physical location ***of*** the microarray substrate and not a location ***on*** the microarray. (Emphasis is added for the Examiner's convenience.) These amendments to Claims 32, 34 and 35 do not narrow the claim scope that was originally intended when Claims 32-35 were filed.

As provided in Applicant's specification at page 26, a physical location of the microarray substrate is a location, such as in a laboratory, or a building that houses a laboratory, where aspects of the hybridization method of the invention might be performed. A 'first location' is a location where an interrogation of the microarray is performed. The first location includes one or both of the physical location where the incubation of the microarray is performed, such as a laboratory or a building housing the laboratory, and further a separate physical location from the laboratory or the building where the incubation is performed. See amended Claim 32. A 'second location' is a physical location that is different from one or both of the first location where the microarray interrogation is performed and the microarray incubation location. See amended Claim 34. The first and second locations may be remote from one another. The remote location is a physically separate location from another location. See amended Claim 35. By 'remote', it is meant in a different building or laboratory from the building or the laboratory where the transmitted data is received, and may be at least one mile, ten miles, or at least one hundred miles apart. As amended, Claims 32-35 make clear that the recited locations are not locations ***on*** the microarray. Support for these amendments can be found in Applicant's specification at page 26, lines 1-9, as mentioned above. Consideration of the amendments to Claims 32, 34 and 35 is respectfully requested.

Claims 20-23, 25, 28 and 31-32 were rejected under 35 USC 102(a) as being anticipated by Goldberg et al. (USPN 6,203,989 B1, March 20, 2001). The Examiner contended that Goldberg et al. teach a method of hybridizing a microarray of oligonucleotides bound to an adsorbed polymer surface on a siliceous substrate with a nucleic acid material, relying on the Abstract, Column 3, lines 33-39, and Column 14, lines 13-30, of USPN 6,203,989 B1.

However, contrary to that contended by the Examiner, Goldberg et al. are silent on hybridizing a microarray of oligonucleotides bound to ***'an adsorbed polymer surface'*** on a siliceous substrate, as presently claimed in Applicant's Claim 20, and Claims 21-23, 25, 28 and 31-32, which are dependent from Claim 20. Still further, Goldberg et al. are silent on an adsorbed polymer surface comprising a polycationic polymer, as claimed in Applicant's Claim 23. While Goldberg et al. do disclose using siliceous substrates at Col. 3, lines 33-39, Goldberg et al. disclose using a silane coating coated on the substrate before any probes are immobilized. It is well known to those skilled in the art that a silane coating is not a polymer coating that is adsorbed on the surface of a substrate. Instead, silane coatings are covalently attached to the substrate surface and moreover, provide a covalently attachment to oligonucleotide probes. In contrast, an adsorbed polymer is adsorbed on a surface by electrostatic interactions with the substrate or other noncovalent attachment means, for example. Moreover, oligonucleotides are immobilized on the surface by electrostatic interactions with the adsorbed polymer as well. Therefore, the disclosure by Goldberg et al. of using silane coatings on a substrate surface does not anticipate the adsorbed polymer surface that is described and claimed by Applicant in Applicant's Claim 20, and Claims 21-23, 25, 28 and 31-31, which are dependent from Claim 20.

It light of the above remarks, Goldberg et al. fail to disclose each and every feature of the present invention in Claims 20-23, 25, 28 and 31-32, such that Goldberg et al. fail to anticipate Claims 20-23, 25, 28 and 31-32. Reconsideration and withdrawal of the 35 USC 102(a) rejection of Claims 20-23, 25, 28 and 31-32 are respectfully requested.

The Examiner further contended that Goldberg et al. disclose at Col. 14, lines 13-41 and Col. 10, lines 6-17, incubating at a hybridization temperature of about 55°C to about 70°C. However, Goldberg et al. actually disclose using a temperature range of 25°C to 70°C, and provide only 35°C and 45°C temperatures as examples. In fact, Examples 1 and 2 of Goldberg et al. illustrate using only a 45°C hybridization temperature in Col. 18, line 63-65 and Col. 20, lines 36-37. Goldberg et al. disclose no specific examples that fall within the high temperature hybridization temperature range, as recited in Applicant's Claim 20. Therefore, while Goldberg et al. disclose a broad hybridization temperature range, Goldberg et al. is only concerned with hybridizations at temperatures within a range of 25°C to 50°C, also known in the art as low temperature hybridizations. In contrast, the present invention is concerned with hybridizations at temperatures within a range of about 55°C to about 70°C, known in the art as high temperature hybridizations. Therefore, one skilled in the art would not find anticipated, or obvious for that matter, a high temperature hybridization protocol between about 55°C to about 70°C using a microarray with an adsorbed polymer surface from the disclosure of Goldberg et al. Goldberg et al. simply fail to disclose or suggest using a high temperature hybridization assay within a range of about 55°C to about 70°C with sufficient specificity, and further fail to disclose or suggest assaying a microarray with an adsorbed polymer surface, such that one skilled in the art would find that a high temperature hybridization assay, according to Claims 20-23, 25, 28 and 31-32, was in the skilled artisan's possession.

Moreover, the Examiner further contended that Goldberg et al. disclose at Col. 14, lines 13-41 and Col. 10, lines 6-17, using a hybridization solution comprising a buffer composition that comprises a non-chelating buffer selected from MES, a pH within a range of 6.4 to 7.5, and a monovalent cation selected from NaCl, in a concentration ranging from about 0.01 M to about 2.0 M. However, Goldberg et al. actually disclose using a variety of buffers, both chelating and non-chelating, in USPN 6,203,989 B1, without distinction for hybridization assays, including phosphate (e.g., SSPE) and TRIS buffers, as well as sulfonate buffers, such as MES and MOPS. See

Column 8, line 61 to Column 9, line 1, which repeats at Column 9, line 65 to Column 10, line 6, and see Column 14, line 5-12 of USPN 6,203,989 B1.

Goldberg et al. disclose using buffers that Applicant specifically discloses should not be used with microarrays having an adsorbed polymer surface in the hybridization solution of the present invention at hybridization temperatures of about 55°C to about 70°C. The present invention is so directed because the present invention is directed to solving a problem in the high temperature assay art, wherein the problem includes surface degradation of adsorbed polymer surfaces on microarray substrates during a hybridization assay and poor hybridization assay results. See Applicant's specification at page 4, lines 1-8, and elsewhere therein, for example, for a statement of the problem solved by the present invention. However, Goldberg et al. provide no disclosure or suggestion to one skilled in the art that certain ones of the disclosed buffers used in high temperature assays (i.e., at or above about 55°C) with adsorbed polymer surfaces can degrade the surface or at least can result in poor assay results.

It is respectfully submitted that the disclosure of Goldberg et al. fails to recognize or appreciate the problem that Applicant has solved for high temperature hybridization assays on adsorbed polymer surface substrates, such that one skilled in the art would not realize the claimed features of Applicant's present invention from the disclosure of Goldberg et al. Further, one skilled in the art faced with a problem of surface degradation or poor hybridization results during high temperature assays of between about 55°C to about 70°C would not look to Goldberg et al. for a solution. Certainly, one skilled in the art would not obtain from the disclosure of Goldberg et al. ('989) the combination of parameters that Applicant presently claims as a solution to the problem in the high temperature assay art without the benefit of the teachings of the present invention.

In light of the above remarks, Goldberg et al. fail to disclose certain aspects of the claimed invention, and further fail to disclose other aspects of the claimed invention with sufficient specificity. In fact, Goldberg et al. essentially teach away

from the present invention, such that one skilled in the art would not determine that non-chelating buffers, which maintain a solution pH within a range of pH 6.4 to 7.5, should be used in high temperature hybridization assays ranging from about 55°C to about 70°C instead of the chelating buffers, also disclosed by Goldberg et al., with a microarray having an adsorbed polymer surface. Moreover, the skilled artisan would not be motivated to use such non-chelating buffers instead of the chelating buffers, also disclosed by Goldberg et al., under the assaying conditions that are claimed by Applicant in Claims 20-23, 25, 28 and 31-32, to obtain satisfactory hybridization results. Again, reconsideration and withdrawal of the rejection of Claims 20-23, 25, 28 and 31-32 are respectfully requested.

Claims 20-25, 28 and 31-35 were rejected under 35 USC 103(a) over Goldberg et al. in view of Reynolds et al. (USPN 6,316,608 B1). The Examiner contended that Goldberg et al. teach the method of Claims 20-22, 25, 28 and 31-32. The Examiner admitted that Goldberg et al. do not teach a method wherein the adsorbed polymer surface comprises a polycationic polymer polyethylenediamine. However, the Examiner contended that Reynolds et al. teach a method wherein the adsorbed polymer surface comprises a polycationic polymer polyethylenediamine, relying on Column 5, lines 22-49 of USPN 6,316,608 B1 of Reynolds et al. The Examiner further contended that it would have been *prima facie* obvious to one having ordinary skill in the art at the invention was made to combine and substitute a method, wherein the adsorbed polymer surface comprises a polycationic polymer polyethylenediamine further comprising the step of transmitting data representing a result of the interrogation and receiving the same at a second location remote from the first location of Reynolds et al. in the nucleic acid hybridization buffer of Goldberg et al. Applicant respectfully traverses this rejection.

As mentioned above, contrary to that contended by the Examiner, Goldberg et al. fail to disclose or suggest using an adsorbed polymer surface anywhere in USPN 6,203,989 B1 of Goldberg et al. A silane coating, as described in Column 14, lines 21-23, of USPN 6,203,989 B1 is not the same as an adsorbed polymer surface, as presently claimed. Moreover, one skilled in the art would not be motivated from the

disclosure of Goldberg et al. to use an adsorbed polymer surface on a siliceous substrate to immobilize oligonucleotides for hybridizing a microarray, as claimed by Applicant, without the teachings of the present invention. Therefore, contrary to that contended by the Examiner, the disclosure of Goldberg et al. does not teach a method including using an adsorbed polymer surface, as claimed in Claims 20-22, 25, 28 and 31-32.

Applicant agrees with the Examiner that Goldberg et al. do not teach a method wherein an adsorbed polymer surface comprises a polycationic polymer polyethylenediamine. In fact, Goldberg et al. do not teach an adsorbed polymer surface at all. This feature of an adsorbed polymer surface comprising a polycationic polymer, such as polyethylenediamine is claimed in a combination of Applicant's dependent Claims 23 and 24, which are ultimately dependent from Claim 20. The Examiner relied on the disclosure of Reynolds et al. to provide support for using an adsorbed polymer surface of polyethylenediamine. However, it is respectfully submitted that Reynolds et al. disclose using 'polyethylenimine' as an example of a 'covalent attachment means' and not as an adsorbed polymer surface. Reynolds et al. do not disclose using 'polyethylenediamine' as 'an adsorbed polymer surface', as the Examiner contended. (Emphasis provided for the Examiner's convenience.)

Reynolds et al. are directed to methods for determining the relative amounts of individual polynucleotides in a complex mixture using hybridization assays with arrays of two or more distinct polynucleotides that are combined prior to assaying and using fluorescence associated with combined polynucleotides to provide a measure of the relative amount. At Column 5, lines 22-49, of USPN 6,316,608 B1, Reynolds et al. disclose how the combined polynucleotides may be stably associated with the substrate. Reynolds et al. disclose using either covalent or noncovalent attachment means. For ***covalent attachment***, the substrate includes or is treated to include chemical groups, such as silylated glass, hydroxyl, carboxyl, amine, aldehyde or sulfhydryl groups. Once the combined polynucleotides are deposited on the substrate surface, they are fixed by covalent attachment means. Examples of ***covalent attachment means for fixing the polynucleotides*** include, at lines 31-40 of Column 5,

drying the combined polynucleotide spots on the array surface, and exposing the surface to a solution of a cross-linking agent, such as glutaraldehyde, borohydride, or any of a number of available bifunctional agents. Alternatively, the ***covalent attachment means for fixing the polynucleotides*** includes using an alkylamino-linker group or by coating the glass slides with ***polyethylenimine*** followed by activation with cyanuric chloride for coupling the oligonucleotides (see lines 35-40 of Column 5). Therefore, contrary to that contended by the Examiner, Reynolds et al. disclosure of using a ***polyethylenimine*** at Column 5, line 38, is part of another example of a cross-linked, ***covalent attachment means*** - and not an example of an adsorbed polymer surface that includes a ***'polycationic polymer polyethylenediamine'***. (Emphasis provided above is for the Examiner's convenience.)

At Column 5, lines 40-49 of USPN 6,316,608 B1, Reynolds et al. do disclose ***noncovalent attachment means*** for attaching the combined polynucleotide to the substrate surface, typically by electrostatic interaction, using a coating of a polycationic polymer, preferably a cationic polypeptide. Reynolds et al. also disclose stably associating the combined polynucleotides to the surface using a combination of covalent and noncovalent means. (Emphasis provided above is for the Examiner's convenience.)

Therefore, Reynolds et al. fail to disclose using a polycationic polymer selected from polyethylenediamine, as contended by the Examiner. In fact, Reynolds et al. fail to disclose examples of polycationic polymers, but for using a cationic polypeptide without a mention of any specific ones.

Moreover, Reynolds et al. fail to disclose or suggest doing high temperature hybridization assays at a hybridization temperature within about 55°C to about 70°C, using a non-chelating buffering agent in a hybridization solution, that maintains solution pH within a range of pH 6.4 - 7.5, and using a monovalent cation in a monovalent cation concentration ranging from about 0.01 M to about 2.0 M, as claimed in Claim 20, and in Claims 21-25, 28 and 31-35, which are ultimately dependent therefrom. Therefore, the lack of disclosure of Reynolds et al. combined

with the lack of, and the insufficient specificity in, the disclosure of Goldberg et al., would not render *prima facie* obvious to one skilled in the art at the time of the present invention to perform hybridization assays, as claimed by Applicant in Claims 20-25, 28 and 31-35, at a high hybridization temperature ranging from about 55°C to about 70°C with a microarray having an adsorbed polymer surface in a hybridization solution comprising in a non-chelating buffer agent that maintains a solution pH within a range of pH 6.4 - 7.5, and a monovalent cation in a monovalent cation concentration ranging from about 0.01 M to about 2.0 M. The benefit of the teachings of the present invention would be necessary to find obviousness therein.

In light of the above, it is respectfully submitted that hindsight would be necessary to find Claims 20-25, 28 and 31-35 obvious. Reconsideration and withdrawal of the rejection under 35 USC 103(a) of Claims 20-25, 28 and 31-35 with respect to Goldberg et al. and Reynolds et al. are respectfully requested.

Claims 20-22, 25-28 and 31-32 were rejected under 35 USC 103(a) over Goldberg et al. in view of Cohen, USPN 6,322,989 B1. The Examiner contended that Goldberg et al. teach the method of Claims 20-22, 25, 28 and 31-32. The Examiner admitted that Goldberg et al. do not teach a method wherein the buffer composition further comprises an ionic surfactant SDS at a concentration ranging from about 0.01% to about 0.2% (w/v). However, the Examiner contended that Cohen teaches a method, wherein the buffer composition further comprises an ionic surfactant SDS at a concentration ranging from about 0.01% to about 0.2% (w/v) at Column 19, lines 46-51 and Column 20, lines 1-5 of USPN 6,322,989 B1. The Examiner contended that it would be *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to combine and substitute a method, wherein the buffer composition further comprises an ionic surfactant SDS at a concentration ranging from about 0.01% to about 0.2% (w/v) in the nucleic acid hybridization buffer of Goldberg et al. Applicant respectfully traverses this rejection.

As mentioned above, contrary to that contended by the Examiner, Goldberg et al. fail to disclose the present invention as claimed in Claims 20-22, 25, 28 and 31-32.

Applicant agrees with the Examiner that Goldberg et al. do not teach a method wherein the buffer composition further comprises an ionic surfactant SDS at a concentration ranging from about 0.01% to about 0.2% (w/v). This feature of the present invention is claimed in a combination of Applicant's dependent Claims 26 and 27, which are ultimately dependent from Claim 20. In fact, Goldberg et al. instead disclose using a ***non-ionic*** surfactant Triton X-100 in a hybridization solution at least at Column 10, line 5 and Column 14, lines 9-12, comprising SSPE; Column 10, lines 12-14 and Column 14, lines 33-35, comprising MES; Column 14, lines 39-41; and Example 2. Moreover, Goldberg et al. provide no warnings or cautions to one skilled in the art with respect to using ***non-ionic*** Triton X surfactants in high temperature hybridization assays with adsorbed polymer surfaces. Applicant's specification at page 20, line 27 - 31 specifically states that Triton X-100® and X-102® are ***non-ionic*** octylphenoxy-polyoxyethylene ethers that are trademarks of Union Carbide. Certain surfactants, including Triton X-100® and X-102®, in low concentrations have a large negative effect on the stability of the adsorbed polymer surface for reasons that are not known. Therefore, Goldberg et al. also essentially teach away from the present invention.

Moreover, Goldberg et al. also fail to disclose or suggest "... and wherein the step of incubating comprises using a hybridization chamber, and the ionic surfactant is provided in an amount sufficient to wet surfaces of the hybridization chamber and loosen bubbles impinged on the surfaces of the hybridization chamber", as recited in part in Claim 26.

USPN 6,322,989 B1 to Cohen is directed to a method of detecting ovarian or breast cancer in a subject using a whole blood sample from the subject that is assayed to form a tumor antigen-antibody complex that is detected. At Column 19, lines 40-51, Cohen disclose using a SSC buffering agent that further comprises 0.1% (w/v) dextran sulfate in a hybridization solution, and further discloses that those skilled in the art will include detergents, such as SDS, chelating agents, such as EDTA and other reagents. At Column 20, lines 1-5, Cohen discloses using a hybridization solution comprising a SSC buffering agent and a wash solution comprising SSC and 0.1%

(w/v) SDS. While Cohen does disclose incubating at a high temperature of 65°C and generally using SDS at Columns 19 and 20, Cohen discloses using the high temperature and SDS with a ***chelating*** buffering agent SSC. Further, Cohen fails to disclose using a microarray having an adsorbed polymer surface and a method of hybridizing the microarray comprising incubating in a hybridization solution comprising a ***non-chelating*** buffering agent, a pH within a range of 6.4 to 7.5, and a monovalent cation in a concentration ranging from about 0.01 M to about 2.0 M. Instead, Cohen discloses using only a buffering agent that is ***not*** non-chelating. Applicant describes the SSC buffering agent as problematic and not recommended in Applicant's specification at least at page 4, lines 1-8, and page 18, lines 1-12, when using adsorbed polymer surfaces in high temperature hybridizations. Therefore, Cohen essentially teaches away from the claimed invention. Still further, Cohen fails to disclose or suggest "... and wherein the step of incubating comprises using a hybridization chamber, and the ionic surfactant is provided in an amount sufficient to wet surfaces of the hybridization chamber and loosen bubbles impinged on the surfaces of the hybridization chamber", as recited in part in Claim 26.

In light of the above, the disclosure of Cohen fails to add to that lacking in the disclosure of Goldberg et al. that which would render Applicant's Claims 20-22, 25-28 and 31-32 *prima facie* obvious to one skilled in the art at the time the invention was made. It is respectfully submitted that one skilled in the art, having the benefit of the combined disclosures of Goldberg et al. and Cohen, would have to pick and choose among a variety of different hybridization conditions disclosed by them, including conditions or parameters that teach away from the claimed invention. The skilled artisan would have to choose among the disclosed conditions and parameters without any motivation or direction from Goldberg et al. and Cohen to pick certain conditions over others to achieve that claimed in the present invention. Therefore, an application of hindsight would be necessary to find obviousness therein. Reconsideration and withdrawal of the rejection of Claims 20-22, 25-28 and 31-32 under 35 USC 103(a) with respect to the combined disclosures of Goldberg et al. and Cohen are respectfully requested.

Claims 20-22, and 25-32 were rejected under 35 USC 103(a) over Goldberg et al. in view of Cohen and further in view of McDonough et al., USPN 6,252,059 B1. The Examiner contended that Goldberg et al. in view of Cohen teach the method of Claims 20-22, 25-28 and 31-32. The Examiner admitted that Goldberg et al. do not teach a method wherein the buffer composition further comprises a monovalent cation LiCl at a concentration greater than or equal to 300 mM. The Examiner further contended that McDonough et al. teach a method, wherein the buffer composition further comprises a monovalent cation LiCl at a concentration greater than or equal to 300 mM, relying on Column 4, lines 2-10 and Column 8, lines 15-50 of USPN 6,252,059 B1 for support of this contention. The Examiner contended that it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to combine and substitute a method, wherein the buffer composition further comprises a monovalent cation LiCl at a concentration greater than or equal to 300 mM of McDonough et al. in the method of Goldberg et al. in view of Cohen. Applicant respectfully traverses this rejection.

For the reasons set forth above, contrary to that contended by the Examiner, Goldberg et al. in view of Cohen fail to make obvious the present invention according to Claims 20-22, 25-28 and 31-32. Applicant agrees that Goldberg et al. fail to teach a method wherein the buffer composition further comprises a monovalent cation LiCl at a concentration greater than or equal to 300 mM. Applicant's claims this feature in dependent Claim 29. Moreover, Claim 29 further recites in part "... the non-chelating buffering agent is 2-[*N*-morpholino]ethanesulfonic acid (MES) ... the pH is within the range of pH 6.6 to 6.8". Claim 29 depends directly from independent Claim 20 and includes all of the limitations recited in Claim 20. As mentioned above, Goldberg et al. in view of Cohen fail to make obvious the present invention according to Claim 20. Since Claim 20 is nonobvious over Goldberg et al. in view of Cohen, then dependent Claim 29 is not obvious with respect to Goldberg et al. in view of Cohen, and further in view of McDonough et al.

USPN 6,252,059 B1 to McDonough et al. is directed to the design and construction of amplification oligonucleotides and probes to the human

immunodeficiency virus type 1 (HIV) to allow detection of HIV nucleic acid in a test sample. At Column 4, lines 2-10 of USPN 6,252,059 B1, McDonough et al. disclose using stringent hybridization conditions including 0.05 M lithium succinate buffer containing 0.6 M LiCl at 60°C. At Column 8, lines 27-50 of USPN 6,252,059 B1, McDonough et al. disclose that target in 50 µl of 10 mM HEPES, 10 mM EDTA, 1% LLS, pH 7.4, was denatured at 95°C for 5 minutes; cooled on wet ice; and 0.04 pmol of probe in 50 µl of 0.1 M lithium succinate buffer, pH 4.7, 2% (w/v) LLS, 1.2 M LiCl, 10 mM EDTA and 20 mM EGTA was added. Hybridization was carried out at 60°C for 10 minutes followed by addition of 300 µl of 0.6 M sodium borate, pH 8.5, 1% Triton X-100 and a second incubation at 60°C for 6 minutes to hydrolyze the AE on unhybridized probe. Samples were cooled in ice water for 1 minute, placed at room temperature for another 3 minutes, and then analyzed.

It is respectfully submitted that while McDonough et al. disclose using greater than 300 mM LiCl in a hybridization solution during a high temperature assay at 60°C, McDonough et al. also disclose using lithium succinate buffer, pH 4.7, and 0.6 M sodium borate, pH 8.5, and 1% Triton X-100, which teach away from the claimed invention. Further, McDonough et al. fail to disclose or suggest using a microarray of oligonucleotides on an adsorbed polymer surface. The pHs used by McDonough et al. are outside the range claimed by Applicant, and the Triton X-100 is a non-ionic surfactant the Applicant recommends against using with adsorbed polymer surfaces in high temperature assays. Moreover, McDonough et al. fail to add to that lacking in the combined disclosures of Goldberg et al. and Cohen to render Applicant's invention obvious to one skilled in the art. Therefore, without the benefit of the teachings of the present invention, one skilled in the art would not be able to pick and choose the features recited in Applicant's Claims 20-22 and 25-32 from the myriad of hybridization solution elements disclosed by Goldberg et al., Cohen and McDonough et al., such that Applicant's invention according to Claims 20-22 and 25-32 would have been *prima facie* obvious at the time the invention was made.

In light of the above, reconsideration and withdrawal of the rejection of Claims 20-22 and 25-32 under 35 USC 103(a) with respect to the combined disclosures of Goldberg et al., Cohen and McDonough et al. are respectfully requested.

Claim 50 is pending, but was not rejected by the Examiner in the pending Office Action. However, the Examiner did not indicate any allowability of Claim 50 either. Applicant respectfully submits that Claim 50 is allowable over Goldberg et al. in view of any combination of Reynolds et al., Cohen and McDonough et al. for at least the same reasons set for above for Claims 20-35.

In summary, Claims 1-57 were pending. Claims 1-19, 36-49 and 51-57 were canceled herein, without prejudice, for being drawn to a non-elected invention. Claims 20-35 and 50 are pending. Claims 20-35 and 50 were rejected in the pending Office Action, however the Examiner made no specific rejection of Claim 50. Applicant has amended Claims 32, 34, 35 and 50. The amendments to the Claims are requested in the attached Appendix that follows below. Claims 20-35 and 50, as amended herein, are in condition for allowance. It is respectfully requested that Claims 20-35 and 50, as amended herein be allowed, and that the application be passed to issue at an early date.

Should the Examiner have any questions regarding the above, please contact Gordon M. Stewart, Attorney for Applicant, Registration No. 30,528 at Agilent Technologies, Inc., telephone number (650) 485-2386.

Respectfully submitted,
NELSON R. HOLCOMB ET AL.

By: Elizabeth E. Leitereg
Elizabeth E. Leitereg
Attorney for Applicant(s)
Registration No. 34,101

The above-referenced attached Appendix follows after this page.